

That which is claimed is:

1. A TRAF-Protein-Binding-Domain (TPBD) polypeptide comprising SEQ ID NO:19, provided said polypeptide is no longer than 213 amino acids.
- 5 2. The TPBD polypeptide of claim 1, further comprising SEQ ID NO:20.
3. The TPBD polypeptide of claim 1, further comprising SEQ ID NO:21.
- 10 4. The TPBD of claim 1, wherein the amino acid sequence of said protein comprises substantially the same sequence as any of SEQ ID NOS:8, 10, 12, 23, 24 or 25.
- 15 5. The TPBD of claim 1, comprising the same amino acid sequence as set forth in any of SEQ ID NOS:8, 10, 12, 23, 24 or 25.
6. A TPBD according to claim 4, wherein said polypeptide is encoded by a nucleotide sequence comprising substantially the same nucleotide sequence as set forth in SEQ ID NOS:7, 9 or 11.
- 20 7. A TPBD according to claim 1, wherein said polypeptide is encoded by a nucleotide sequence comprising the same sequence as set forth in SEQ ID NOS:7, 9 or 11.
- 25 8. An isolated anti-TPBD antibody having specific reactivity with a TPBD according to claim 1.

Ans  
A1

Sub A1  
9. Antibody according to claim 8, wherein said antibody is a monoclonal antibody.

10. A cell line producing the monoclonal antibody of claim 9.

5 Sub A2  
11. An antibody according to claim 8, wherein said antibody is a polyclonal antibody.

12. Isolated nucleic acid encoding a TRAF protein binding domain (TPBD), or functional fragments thereof, selected from:

10 (a) DNA encoding the amino acid sequence set forth in SEQ ID NOS:8, 10 or 12, 23, 24 or 25, or

(b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein  
15 said DNA encodes biologically active TPBD, or

(c) DNA degenerate with (b), wherein said DNA encodes biologically active TPBD.

13. A nucleic acid according to claim 12, wherein said nucleic acid hybridizes under high  
20 stringency conditions to the TPBD coding portion of any of SEQ ID NOS:7, 9 and 11.

14. A nucleic acid according to claim 12, wherein the nucleotide sequence of said nucleic acid is substantially the same as set forth in any of SEQ ID  
25 NO:7, 9 and 11.

15. A nucleic acid according to claim 12, wherein the nucleotide sequence of said nucleic acid is the same as that set forth in any of SEQ ID NOS:7, 9 and 11.

16. A nucleic acid according to claim 12, wherein said nucleic acid is cDNA.

17. A vector containing the nucleic acid of claim 12.

5 18. Recombinant cells containing the nucleic acid of claim 12.

10 19. An oligonucleotide comprising at least 15 nucleotides capable of specifically hybridizing with a the nucleotide sequence set forth in any of SEQ ID NOs:7, 9 and 11.

20. An oligonucleotide according to claim 19, wherein said oligonucleotide is labeled with a detectable marker.

15 21. An antisense-nucleic acid capable of specifically binding to mRNA encoded by said nucleic acid according to claim 12.

22. A kit for detecting the presence of the TRAF cDNA sequence comprising at least one oligonucleotide according to claim 20.

20 23. A composition comprising an amount of the antisense-nucleic acid according to claim 21 effective to inhibit expression of a human TPBD and an acceptable hydrophobic carrier capable of passing through a cell membrane.

25 24. A method for expression of a TPBD, said method comprising culturing cells of claim 18 under conditions suitable for expression of said TPBD.

✓ 25. A method for identifying nucleic acids encoding a mammalian TPBD, said method comprising contacting a sample containing nucleic acids with an oligonucleotide according to claim 19, wherein said  
5 contacting is effected under high stringency hybridization conditions, and identifying compounds which hybridize thereto.

✓ 26. A method for detecting the presence of a human TPBD in a sample, said method comprising contacting  
10 a test sample with an antibody according to claim 8, detecting the presence of an antibody:TPBD complex, and therefor detecting the presence of a human TPBD in said test sample.

✓ 27. Single strand DNA primers for  
15 amplification of TPBD nucleic acid, wherein said primers comprise a nucleic acid sequence derived from the nucleic acid sequences set forth as SEQ ID NOs:7, 9 and 11.

28. A method of modulating a TNF family receptor comprising contacting a cell with an agent that  
20 modulates the activity of a TDBP-containing protein, wherein said TNF family receptor is selected from TNFR1, TNFR2, CD27, CD30, CD40, 4-1BB, Ox40, LT- $\beta$ R, Fas, DR3, DR4, DR5, HVEM, LMP-1, IL-1R, and a member of the TNF receptor family that does not comprise a death domain.

25 29. The method of claim 28 wherein said TNF family receptor is selected from TNF-R2, CD40, LT- $\beta$ R, NGFRp75 and DR4.

30. A method of modulating a TRAF protein comprising contacting a cell with an agent that modulates the activity of a TDBP-containing protein.

31. The method of claim 30 wherein said TRAF protein is selected from human TRAF1, human TRAF2, human TRAF3, human TRAF4, human TRAF5 and human TRAF6.

32. A method of modulating a TRAF-associated protein comprising contacting a cell with an agent that modulates the activity of a TDBP-containing protein, wherein said TRAF-associated protein is selected from TRADD, FADD, I-TRAF, TRIP, A20, c-IAP1, c-IAP2, Casper, RIP, RIP2, NIK, Peg3, GCK, NIK, ASK1 and IRAK.

33. A method of modulating the activity of NF- $\kappa$ B or cJun N-terminal kinase comprising contacting a cell with an agent that modulates the activity of a TDBP-containing protein.

34. The method of claim 33 wherein said NF- $\kappa$ B activity is modulated.

35. A method of modulating a cell process comprising contacting a cell with an agent that modulates the activity of a TDBP-containing protein, wherein said cell process is selected from apoptosis, cell proliferation, cell adhesion, cell stress responses and B cell immunoglobulin class switching.

36. A method for modulating the activity of an oncogenic protein, comprising contacting said oncogenic proteins with a substantially pure TPBD, or an oncogenic protein-binding fragment thereof.

37. A method of identifying an effective agent that modulates the association of a TPBD with a TRAF protein, comprising the steps of:

5 a) contacting said TPBD and TRAF proteins, under conditions that allow said TPBD and TRAF proteins to associate with an agent suspected of being able to modulate the association of said TPBD and TRAF proteins; and

10 b) detecting the modulated association of said TPBD and TRAF proteins, wherein said modulated association identifies an effective agent.

15 38. The method of claim 37, wherein said altered association is detected by measuring the activity of NF- $\kappa$ B.

39. The method of claim 37, wherein said altered association is detected by measuring the activity of c-Jun N-terminal kinase.

20 40. The method of claim 37, wherein said effective agent is a drug.

41. The method of claim 37, wherein said effective agent is a protein.

25 42. A method for modulating an activity mediated by a TPBD, said method comprising contacting said TPBD with an effective, modulating amount of an agent identified by claim 37.

✓ 43. The method of claim 42, wherein said modulated activity is selected from the group consisting of: binding of TPBD to a TNRF; binding of TPBD to a TRAF protein; binding of TPBD to a TRAF-associated protein;

5 NF-κB activity, c-Jun N-terminal kinase activity, apoptosis activity, cell proliferation activity, cell adhesion, cell stress response activity and B cell immunoglobulin class switching activity.

✓ 44. A method of modulating the level apoptosis  
10 in a cell, comprising the steps of:

a) introducing a nucleic acid molecule encoding a TPBD into the cell; and

b) expressing said TPBD in said cell, wherein the expression of said TPBD modulates apoptosis  
15 in said cell.

✓ 45. A method of modulating class switching in a B cell, comprising introducing an antisense nucleotide sequence into the cell, wherein said antisense nucleotide sequence specifically hybridizes to a nucleic acid  
20 molecule encoding a TPBD, wherein said hybridization reduces or inhibits the expression of said TPBD in said cell.

46. A therapeutic composition comprising a compound selected from a TPBD, or functional fragment  
25 thereof, a TPBD modulating agent identified according to claim 37, or an anti-TPBD antibody; and a pharmaceutically acceptable carrier.

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A3

47. A method of treating a pathology characterized by abnormal cell proliferation or abnormal immunoglobulin class-switching, said method comprising administering an effective amount of the composition  
5 according to claim 46.

✓ 48. A method of diagnosing a pathology characterized by an increased or decreased level of a TPBD in a subject, comprising the steps of:

- 10 a) obtaining a test sample from the subject;
- b) contacting said test sample with an agent that can bind said TPBD under suitable conditions, which allow specific binding of said agent to said TPBD; and
- 15 c) comparing the amount of said specific binding in said test sample with the amount of specific binding in a control sample, wherein an increased or decreased amount of said specific binding in said test sample as compared to said control sample is diagnostic
- 20 of a pathology.

✓ 49. The method of claim 48, wherein said agent is an anti-TPBD antibody, a TNF family receptor, a TRAF protein, or a TRAF-associated protein.

25 50. A method of modulating the level of apoptosis in a cell, comprising contacting the cell with an agent that effectively alters the association of TPBD with a TRAF-associated-protein in the cell, or that effectively alters the activity of NF- $\kappa$ B or JNK in the cell.



51. A chimeric protein comprising the TPBD of claim 1.

52. A chimeric TPBD-containing protein, comprising the sequence SEQ ID NO:19, provided said  
5 chimeric protein is not naturally occurring.

53. A chimeric TPBD-containing protein, comprising:

- (a) the sequence SEQ ID NO:19; and
- (b) a sequence from a heterologous protein.

10 54. The chimeric TPBD-containing protein of claim 53, further comprising SEQ ID NO:20.

55. The chimeric TPBD-containing protein of claim 53, further comprising SEQ ID NO:21.

15 56. The chimeric protein of claim 53 further comprising a RING finger domain.

57. An isolated TPBD-containing TRAF protein, or fragment thereof, comprising the sequence SEQ ID NO:19, provided said TRAF domain containing protein does not consist of the sequence SEQ ID NOs:2, 4 or 6.

20 58. The TPBD-containing TRAF protein of claim 57, further comprising the sequence SEQ ID NO:20.

59. The TPBD-containing TRAF protein of claim 57, further comprising the sequence SEQ ID NO:21.

25 60. A TPBD-containing polypeptide comprising the sequence SEQ ID NO:20, provided said polypeptide is no longer than 213 amino acids.

61. The method of claim 37, wherein said agent modulates TPDB association with a TRAF protein, or TPBD association with a TNF family receptor, or TPBD association with a TRAF-associated protein.

5 62. A method of modulating TPBD:TRAF protein interactions comprising contacting a TPBD with the agent of claim 61.

63. The method of claim 37, wherein said agent modulates JNK activity.

10 64. The method of claim 37, wherein said agent modulates NF- $\kappa$ B activity.

65. A method of modulating class switching comprising contacting a cell with a compound selected from the group consisting of: a TPBD or functional  
15 fragment thereof, an agent identified according to claim 28, and an anti-TPBD antibody.

66. A method of diagnosing cancer or monitoring cancer therapy comprising contacting a test sample from a patient with the antibody of claim 8.

20 67. A method of assessing prognosis of patients with cancer comprising contacting a test sample from a patient with the antibody of claim 8.

68. An effective agent that binds a TRAF ☒  
protein binding site of TPBD.

25 69. An effective agent that modulates the association of TPBD with a TNF family receptor or a TRAF protein, TRAF protein or a TRAF-associated protein.

70. The agent of claim 69, wherein said TNF family receptor is TNF-R2, said TRAF protein is human TRAF6 and said TRAF-associated protein is I-TRAF.

71. The agent of claim 69, wherein said TNF family receptor is CD40, said TRAF protein is human TRAF2 and said TRAF-associated protein is I-TRAF.

72. The agent of claim 69, wherein said effective agent inhibits the association of said TPBD with said TNF family receptor or a TRAF protein, TRAF protein or a TRAF-associated protein.

73. The agent of claim 69, wherein said effective agent increases the association of said TPBD with said TNF family receptor or a TRAF protein, TRAF protein or a TRAF-associated protein.

add AS

add TNF-R

CD40

binds both

TRAF6  
I-TRAF

TRAF2  
I-TRAF

TNF-R  
TRAF

TRAF-TRAF assoc protein